T7 RNA Polymerase

Product Number: GMP-T702

Shipping and Storage

-20℃

Components

Component	GMP-T702
T7 RNA Polymerase(200U/μl)	25μl
T7 RNA Polymerase(200U/μl)	1ml
T7 RNA Polymerase(200U/μl)	10ml
T7 RNA Polymerase(200U/μl)	50ml

Description

As a biomolecule, mRNA can be synthesized on a large scale through in vitro transcription (IVT), and the T7 promoter is currently one of the most efficient promoters. Therefore, using T7 RNA polymerase for in vitro transcription can easily and quickly obtain a large number of RNA molecules. As one of the byproducts of in vitro transcription, dsRNA can be recognized by corresponding nucleic acid receptors, triggering a natural immune inflammatory response that seriously affects the effectiveness of mRNA vaccines. Therefore, strict control is required during the production process. The T7 RNA polymerase obtained through molecular evolution modification of this product can significantly reduce the content of dsRNA in transcription products and decrease the immunogenicity of synthesized mRNA.

This product is a GMP grade recombinant T7 RNA polymerase expressed through large-scale fermentation of Escherichia coli. It is produced using pharmaceutical grade raw materials and strictly controls host protein residues, nucleic acid residues, etc. It complies with GMP standards for product production and quality management regulations to ensure traceability of the production process and all raw materials.

Item	Standard
Appearance	Clear and transparent solution
Identify	Positive
Visible Particles	Meet the specification
pH value	7.3-7.7
Activity	180U/μl-220U/μl
Purity	≥95%
Protein content	Conforms
Endonuclease Residues	The degradation of substrate was ≤10%
Exonuclease Residues	The degradation of substrate was ≤10%
RNase Residues	The degradation of substrate was ≤10%
Bacterial endotoxin	< 5EU/ml
Exogenous DNA residue	≤ 100pg/mg
Residual bacterial protein	≤ 50ppm
Mycoplasma	Negative
Heavy metal	≤ 10ppm
Microbial limit	The total number of aerobic bacteria should not exceed 1cfu/10ml, and the total number of mold and yeast should not exceed 1cfu/10ml

Complying to following regulations

1. ISO 9001:2015, certified facility.
2. GMP Appendix – Cellular therapeutic product National Medical Products Administration.
3. General Introduction to Human Gene Therapy - Chinese Pharmacopoeia 2020, National Pharmacopoeia Commission.
4. USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
5. USP Chapter <92>, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.
6. Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

Features

It has high specificity for the T7 promoter.

Source	E. carrying bacteriophage T7RNA polymerase gene coli
Storage buffer	50mM Trizma base; 100mM NaCl; 1mM EDTA; 20mM β-ME; 50% (v/v) Glycerol; 0.1% Triton X-100; pH 7.5
Storage	-20℃±5℃
Unit definition	The enzyme amount required to incorporate 1nmol of [3H] GMP into an acid insoluble precipitate within 1 hour at 37 ℃ and pH 8.0 is defined as 1 active unit

Application

1. Synthesize single stranded RNA for the preparation of mRNA vaccines and other applications.
2. Synthesize highly specific RNA probes.
3. Synthesize siRNA precursor.
4. Preparation of RNA splicing precursors.
5. Synthesize capped RNA using cap analogues.

Note

1. Template efficiency and incubation time:
   The yield of different templates may vary depending on the sequence, structure, length, purity of the template, as well as the sequence and length of specific RNA polymerase promoters. Pollutants that affect transcription yield include ribonucleases or contaminants such as phenol, trace metals, and SDS.
2. Optimized response:
   The recommended reaction conditions can be applied to the in vitro transcription of most templates, but for some templates, the yield can be improved by extending the reaction time (4-hour overnight reaction) and increasing the amount of template used.
3. Maintain RNase free environment:
   3.1. Use RNase free tubes and pipettes;
   3.2. Gloves should be worn when handling kit components or samples containing RNA, and gloves should be changed frequently, especially when in contact with potential sources of RNase contamination such as door handles, pens, pencils, and human skin.
   3.3. When not in use, all reagents should be sealed properly. During the incubation process, seal all test tubes containing RNA.
4. When configuring the reaction system, 0.5μl RNase Inhibitor, GMP Grade (catalog number: GMP-RI01) can be added.
5. Due to the presence of spermidine in the 10×Translation Buffer, it will form a precipitate with the template DNA at low temperatures. When preparing the reaction solution, it needs to be done at room temperature. Adjust the order of component addition, calculate the system, add water, buffer, and NTP first, and finally add the template and enzyme.

Template preparation

Linearized plasmids, PCR products, or synthesized DNA fragments with T7 promoter can be used as in vitro transcription templates for T7 RNA polymerase, which can be dissolved in TE buffer or RNase free water.

1. Plasmid template (it is recommended to add 1μg linearized plasmid as a template for each reaction)
2. The plasmid with T7 promoter can serve as a transcription template, and the linearization and purity of the plasmid can affect the yield of transcription and the integrity of RNA. Due to the lack of effective termination, circular plasmids can transcribe RNA products of different lengths. In order to obtain RNA of a specific length, the plasmid must be completely linearized. Linearized plasmids should ensure that the double stranded end is flat or the 5 'end is a protruding structure.
3. PCR product template (recommended to add 0.1μg~1μg as a template for each reaction)
4. The PCR product with T7 promoter can serve as an in vitro transcription template. Add the T7 promoter to the 5 'end of the upstream primer of the sense chain during PCR amplification of the template. The PCR product is purified and used as a template.
5. The synthesized DNA template (recommended to add 0.1μg~0.5μg as a template for each reaction) can also serve as a template for in vitro transcription of DNA fragments containing the T7 promoter.

Protocol

1. In vitro transcription
   1.1. After melting each component, mix them evenly and briefly centrifuge to collect them at the bottom of the tube. Store them on ice for later use.
   1.2. Add the following components at room temperature:
   Note: After calculating the system, first add water, buffer, and NTP, and finally add the template and enzyme.

Transcription system:

Component	Volume
10×Transcription Buffer, GMP Grade	2μl
ATP/GTP/CTP/UTP Mix	7.5mM (Na-NTP)-10mM (Tris-NTP)
Template DNA	500ng-1μg
T7 RNA Polymerase 2.0, GMP Grade	50-200U
RNase Free Water	Up to 20μl

Co transcriptional system:

Component	Volume
10×Transcription Buffer, GMP Grade	2μl
ATP/GTP/CTP/UTP Mix	7.5mM(Na-NTP-10mM (Tris-NTP)
Cap1 GAG	7.5mM(Na-NTP-10mM (Tris-NTP)
Template DNA	500ng-1μg
T7 RNA Polymerase 2.0, GMP Grade	50-200U
RNase Free Water	Up to 20μl

Note: 1) Due to the salt type of NTP affecting the yield of transcription and co transcription, it is recommended to choose a final concentration of 7.5mM for NTP reaction using Na salt, and the yield can reach 100-160μg; If Tris salt is used for NTP, it is recommended to choose a final concentration of 10mM for the reaction, and the yield can reach 180-220μg.

2) RNase inhibitor can be added to the reaction system at a concentration of 1U/μl to prevent RNase contamination.

3) The template DNA should be RNaseA Free and of high purity, with a recommended OD260/280 of 1.8-2.0.

   1.3. Gently mix the components with a pipette and collect them by centrifugation briefly. Incubate at 37 °C for 2-3 hours.
   Note: To avoid the impact of evaporation on the reaction system, it is recommended to conduct the reaction in a PCR instrument. The reaction time can be adjusted appropriately according to the size of the product fragment. For example, when synthesizing RNA smaller than 0.3kb, the reaction can be extended to 4 hours or longer. Overnight reaction for 16 hours will not affect the quality of the product.

1.4. Add 2-4U of DNase I to the reaction system, incubate at 37°C for 15 minutes, and digest the transcribed DNA template. (Optional)

Note: Compared to the product RNA, the content of template DNA is very low and generally does not need to be removed. It can also be digested with DNase I.

1.5. The synthesized RNA can be used for downstream experiments after electrophoresis analysis and purification.

Note: The product concentration is extremely high, and it needs to be diluted with RNAse free water before testing.

2. Product purification

2.1. Purification of lithium chloride

2.1.1. Add 30μl of Lithium Chloride Preparation Solution (7.5 M Lithium Chloride, 50 mM EDTA) and 30μl of RNase Free Water (note: effective precipitation cannot be obtained by this method when RNA is less than 300nt or concentration is less than 100ng/μl) to a volume of 20μl. The precipitation effect is best when RNA concentration is greater than 400ng/μl. When the concentration of transcription products is low, between 100-400ng/μl, there is no need to dilute with water, and 30μl Lithium Chloride Precipitation Solution can be directly used for precipitation;

2.1.2. Mix well and place at -20°C for at least 30 minutes.

2.1.3. Centrifuge at 12000rpm for 15 minutes, remove the supernatant, and collect the precipitate.

2.1.4. Wash three times with pre cooled 70% ethanol.

2.1.5. Detection after RNase Free Water reconstitution.

2.2. Column purification

Column purification can remove proteins and free nucleotides.

Dilute the product to 100μl by adding 80μl RNase free water before purification, and then purify according to the column purification instructions.

Note: Due to the high RNA production, in order to avoid exceeding the carrying capacity of the binding column, please estimate the required number of columns.

2.3. Magnetic bead purification

Magnetic bead purification can remove proteins and free nucleotides.

Purify according to the instructions for magnetic bead purification.

2.4. Phenol/chloroform purification method

Phenol/chloroform extraction can remove proteins and most free nucleotides.

2.4.1. Dilute the product to 180μl by adding 160μl of RNase free water.

2.4.2. Add 20μl of 3M sodium acetate (pH 5.2) to the diluted product and mix thoroughly with a pipette.

2.4.3. Add 200μl of phenol/chloroform mixture (1:1) for extraction, centrifuge at 10000 rpm for 5 minutes at room temperature, and transfer the upper solution (aqueous phase) to a new RNase free EP tube.

2.4.4. Add chloroform with an equal volume to water for extraction twice, and collect the upper aqueous phase.

2.4.5. Add 2 times the volume of anhydrous ethanol and mix well. Incubate at -20°C for at least 30 minutes and centrifuge at 15000 rpm at 4°C for 15 minutes.

2.4.6. Discard the supernatant and add 500μl of pre cooled 70% ethanol to wash the RNA precipitate. Centrifuge at 15000 rpm at 4°C and discard the supernatant.

2.4.7. Open the lid and dry for 2 minutes. Add 20-50μl of RNase free water or other buffer solution to dissolve the RNA precipitate.

2.4.8. Store at -70°C.

3. RNA quantification

3.1. UV absorption method: Free nucleotides can affect the accuracy of quantification. Please perform RNA purification before using this method.

3.2. Dye method: RiboGreen dye is used for RNA quantification, and free nucleotides do not affect quantification. It can accurately quantify RNA in purified or unpurified reaction products.

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FAQ

1. How to choose restriction endonucleases when linearizing plasmid templates?

   Plasmids with promoters can serve as transcription templates, and the linearization and purity of plasmids can affect transcription yield and RNA integrity. Due to the lack of effective termination, circular plasmids can transcribe RNA products of different lengths. In order to obtain RNA of specific lengths, the plasmid must be completely linearized, and the linearized plasmid must ensure that the double stranded end is flat or the 5 'end is a protruding structure. Therefore, it is necessary to select type II restriction endonucleases that can produce prominent structures at the end or 5 'end, and the recognition site of the enzyme is a rare site.

2. Is there a requirement for the purity of transcription templates?

   The template DNA should be RNaseA Free and of high purity, with a recommended OD260/280 of 1.8-2.0.

3. Do transcription templates need to be removed?

   It is best to add DNase I after transcription to remove the template.

4. Low transcript production or transcription failure:

   Suggest creating a control group and an experimental group. If the yield of the control group is low, please contact us. If the yield of the control group experiment is normal but the yield of the experimental group is low, there may be a quality problem with the template itself that leads to the low yield. Please try the following solution to solve it:

   4.1. The experimental template contains components that inhibit the reaction. It is recommended to purify the template again to determine the quantification and integrity of the template;

   4.2. Regarding the issue with the experimental template sequence, it is recommended to extend the reaction time at 37 °C, increase the amount of template input, or try other promoters and RNA polymerases.

5. Low yield of short transcription products:

   When the transcription product is less than 0.3kb, prolonging the reaction time or increasing the template amount can increase RNA production.

6. Product electrophoresis tailing phenomenon:

   6.1. The experimental operation process was contaminated by RNase;

   6.2. DNA template contaminated by RNase;

   Suggest re purifying the template DNA, and pay attention to RNase contamination control during all experimental processes.

7. RNA product fragment larger than expected:

   The plasmid template is not fully linearized or has a prominent structure at the 3 'end of the sense strand. It is recommended to re linearize the plasmid template to ensure complete linearization. For linearized plasmids, please ensure that the double strand has a flat end or a prominent structure at the 5' end;

   RNA has an incompletely denatured secondary structure, and the denatured gel is replaced to detect RNA products.

8. The RNA product fragment is smaller than expected:

   8.1. The template sequence includes a termination sequence similar to T7 RNA polymerase, which leads to premature termination of transcription. It is recommended to try replacing the RNA polymerase;

   8.2. Advanced structures are formed in the template, and it is recommended to try adding SSB protein;

   8.3. RNase contamination.

Related Products

Product Number	Product Name
GMP-M062	Vaccinia Capping Enzyme, GMP Grade
GMP-T701	T7 RNA Polymerase, GMP Grade
GMP-M072	mRNA Cap 2´O Methyltransferase, GMP Grade
GMP-RI01	RNase Inhibitor, GMP Grade
GMP-M012	Poly(A) Polymerase, GMP Grade

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GMP-DI05	DNase I Recombinant GMP grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade (ppase)
GMP-E131	T7 High Yield RNA Transcription kit, GMP Grade